



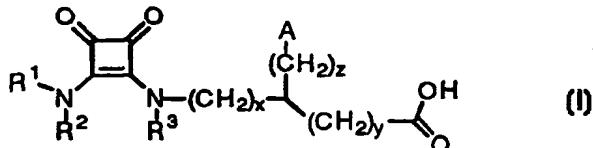
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(54) Title: 3,4-DIAMINO-3-CYCLOBUTENE-1,2-DIONE DERIVATIVES WHICH INHIBIT LEUKOCYTE ADHESION MEDIATED BY VLA-4



(57) Abstract

Compounds of formula (I) which inhibit leukocyte adhesion mediated by interaction of the $\alpha 4\beta 1$ integrin (VLA-4) with its counterreceptor VCAM-1, and their use for the treatment of inflammatory and autoimmune diseases.

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**3,4-DIAMINO-3-CYCLOBUTENE-1,2-DIONE DERIVATIVES WHICH
INHIBIT LEUKOCYTE ADHESION
MEDIATED BY VLA-4**

5 Field of the Invention

This invention relates to novel N-substituted 3,4-diamino-3-cyclobutene-1,2-dione derivatives which inhibit leukocyte adhesion mediated by interaction of the $\alpha_4\beta_1$ integrin (VLA-4) with its counterreceptor VCAM-1, and their use for the treatment of inflammatory and autoimmune diseases.

10

Background of the Invention

VLA-4 (also referred to as $\alpha_4\beta_1$ integrin and CD49d/CD29), first identified by Hemler and Takada (Hemler and Takada, *European Patent Application, Publication No. 330, 506*, published August 30, 1989) is a member of the β_1 integrin family of 15 cell surface receptors, each of which comprises two subunits, an α_4 chain and a β_1 chain. There are at least nine β_1 integrins, all sharing the same β_1 chain and each having a distinct α_4 chain. These nine receptors all bind a different complement of the various cell matrix molecules such as fibronectin, laminin and collagen. VLA-4, for example, binds to fibronectin. VLA-4 is unique among β_1 integrins in that it also 20 binds non-matrix molecules that are expressed by endothelial and other cells. These non-matrix molecules include VCAM-1, which is expressed on cytokine-activated human umbilical vein endothelial cells in culture. Distinct epitopes of VLA-4 are responsible for fibronectin and VCAM-1 binding activities and each activity has been shown to be inhibited independently (Elices, et al., *Cell*, 60:577-584 (1990)).

25

Intercellular adhesion mediated by VLA-4 and other cell surface receptors is associated with a number of inflammatory responses. At the site of an injury or other inflammatory stimulus, activated vascular endothelial cells express molecules that are adhesive for leukocytes. The mechanics of leukocyte adhesion to endothelial cells 30 involves, in part, the recognition and binding of cell surface receptors on leukocytes to the corresponding cell surface molecules on endothelial cells. Once bound, the leukocytes migrate across the blood vessel wall to enter the injured site and release

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chemical mediators to combat infection. For reviews of adhesion receptors of the immune system, see, for example, Springer (Springer, *Nature*, 346:425-434 (1990)) and Osborn (Osborn, *Cell*, 62:3-6 (1990)).

5 Inflammatory brain disorders, such as multiple sclerosis (MS) and meningitis, are examples of central nervous system disorders in which the endothelium / leukocyte adhesion mechanism results in destruction to otherwise healthy brain tissue. Large numbers of leukocytes migrate across the blood brain barrier (BBB) in subjects with these inflammatory diseases. The leukocytes release toxic mediators
10 that cause extensive tissue damage resulting in impaired nerve conduction and paralysis.

In other organ systems, tissue damage also occurs via an adhesion mechanism resulting in migration or activation of leukocytes. For example, it has been shown
15 that the initial insult following myocardial ischemia to heart tissue can be further complicated by leukocyte entry to injured tissue causing still further insult (Vedder, et al., *Surgery*, 106:509 (1989)). Other inflammatory conditions mediated by an adhesion mechanism include asthma (Pretolani, et al., *J. Exp. Med.*, 180:795 (1994); Abraham, et al., *Clin. Invest.*, 93:776 (1994); Mulligan, et al., *Immunology*, 150:2407
20 (1993)), Alzheimer's disease, atherosclerosis (Cybulsky, et al., *Science*, 251:788 (1991); Li, et al., *Atheroscler. Thromb.*, 13:197 (1993)), AIDS dementia (Sasseville, et al., *Am. J. Path.*, 144:27 (1994)), diabetes (Yang, et al., *Proc. Nat. Acad. Science (USA)*, 90:10494 (1993); Burkly, et al., *Diabetes*, 43:526 (1994); Baron, et al., *J. Clin. Invest.*, 93:1700 (1994)), inflammatory bowel disease (Hamann, et al.,
25 *Immunology*, 152:3238 (1994)), multiple sclerosis (Yednock, et al., *Nature*, 356:63 (1992); Baron, et al., *J. Exp. Med.*, 177:57 (1993)), rheumatoid arthritis (van Dinther-Janssen, et al., *Annals. Rheumatic Dis.*, 52:672 (1993); Elices, et al., *J. Clin. Invest.*, 93:405 (1994); Postigo, et al., *J. Clin. Invest.*, 89:1445 (1991)), tissue transplantation (Paul, et al., *Transpl. Proceed.*, 25:813 (1993)), and tumor metastasis (Okahara, et al.,
30 *Can. Res.*, 54:3233 (1994); Paavonen, et al., *Int. J. Can.*, 58:298 (1994); Schadendorf, et al., *J. Path.*, 170:429 (1993)).

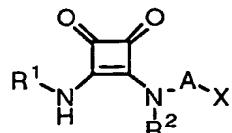
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Because of the significance of VLA-4 in inflammatory and autoimmune conditions, it is desirable to test for the presence of VLA-4 in biological samples and for compounds which inhibit cell adhesion.

Individually, each receptor/ligand interaction is rapidly reversible; however, 5 during the process of cell adhesion, multiple $\alpha_4\beta_1$ integrin receptors on one cell engage multiple VCAM-1 ligands on another cell, and together provide a strong and stable adhesive bond. In order to prevent cell adhesion, small molecule inhibitors of $\alpha_4\beta_1$ integrin must achieve a high degree of receptor occupancy for disruption of a significant number of these adhesive interactions. Furthermore, due to the 10 multivalency of the adhesive interaction, inhibitory compounds exhibit a very steep titration curve, since inhibition begins with 85-90% receptor occupancy and is complete when 95-100% of the receptors are occupied. With such a narrow dynamic range there is considerable assay to assay variation in cell-based adhesion studies. An assay which can detect the presence of a single VCAM-1 molecule with a single 15 receptor and thus prevent assay to assay variation is desired.

N-substituted 3,4-diamino-3-cyclobutene-1,2-dione derivatives have been taught. Japanese Patent JP05229999 A2 930907 discloses cyclobutenediones which are symmetrically disubstituted with α -amino acids.

U.S. Patent No. 5,168,103 issued December 1, 1992, and assigned to 20 American Home Products, describes cyclobutenedione derivatives having formula (2)



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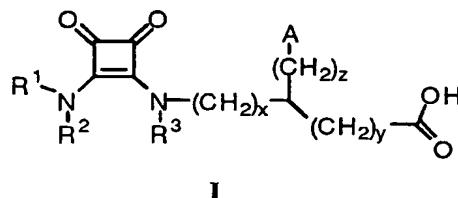
where A is alkylene or alkenylene. These compounds are taught to be useful as N-methyl-D-aspartate antagonists.

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Description of Invention

This invention provides novel compounds of Formula I

5



wherein R¹ is alkyl, aryl, heteroaryl, aralkyl or heteroaralkyl;
 R² is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl; or
 10 R¹ and R² may be taken together to form a saturated or unsaturated heterocycloalkyl;
 R³ is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;
 A is aryl or heteroaryl; and
 x, y and z are independently 0, 1, 2, 3,
 15 or a pharmaceutical salt thereof.

In some preferred embodiments of the present invention R¹ is alkyl of 1 to 10 carbon atoms, aralkyl of 7 to 11 carbon atoms, or heteroaralkyl of 7 to 11 members having 1 to 3 heteroatoms. In still more preferred embodiments of the present 20 invention R¹ is straight chain alkyl of 4 to 8 carbon atoms, benzyl, benzhydryl, phenethyl, pyridylmethyl or pyridylethyl.

R² is preferably hydrogen, alkyl of 1 to 10 carbon atoms or aralkyl of 7 to 11 carbon atoms. More preferably R² is hydrogen, alkyl of 1 to 6 carbon atoms, benzyl or naphthylmethyl.

25 Alternatively, when R¹ and R² are taken together, they preferably form a substituted heterocycloalkyl of 5 to 7 members having 1 to 3 heteroatoms selected from N, O and S.

A is preferably substituted or unsubstituted aryl. When A is substituted the substituent is preferably selected from -NR¹COR⁵, -OCONR⁶R⁷ or -O(CH₂)ₘNR⁶R⁷

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wherein R¹ is hydrogen or alkyl of 1 to 3 carbon atoms, R⁵ is substituted or unsubstituted aryl, heteroaryl or heterocycloalkyl, R⁶ and R⁷ are independently, hydrogen or alkyl of 1 to 3 carbon atoms, or R⁶ and R⁷, taken together may form a substituted heterocycloalkyl, and m is an integer from 1 to 6.

5 In some embodiments of the present invention it is preferred that x and y are 0 and z is 1. R³ is preferably hydrogen in some aspects of the invention.

For purposes of defining preferred substituted heterocycloalkyl, preferred substituents are alkyl of 1 to 3 carbon atoms, aryl, -COR⁸ or -COOR⁹ wherein R⁸ is alkyl of 1 to 3 carbon atoms, aryl of 5 or 6 carbon atoms or aralkyl of 6 or 7 carbon atoms, and R⁹ is hydrogen, alkyl of 1 to 3 carbon atoms, aryl of 5 or 6 carbon atoms or aralkyl of 6 or 7 carbon atoms.

In some embodiments of the present invention R¹ is alkyl, aralkyl or heteroaralkyl, A is phenyl, x and y are 0, and z is 1.

15 More preferred compounds of the present invention are the following compounds:

[2-(Benzylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine;
[2-(benzhydrylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine;
2-{2-[2-(1H-Indol-3-yl)-ethylamino]-3,4-dioxo-cyclobut-1-enylamino}-L-
20 phenylalanine;
{3,4-Dioxo-2-[(pyridin-3-ylmethyl)-amino]-cyclobut-1-enyl}-L-phenyl-
alanine;
[2-(Benzyl-hexyl-amino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine;
(2-Dibenzylamino-3,4-dioxo-cyclobut-1-enylamino)-L-phenylalanine;
25 (S)-2-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-phenyl-propionic
acid;
(S)-2-[2-(Hexyl-naphthalen-2-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl-
amino]-3-phenyl-propionic acid;
30 (S)-2-{2-[(4-Dimethylamino-benzyl)-hexyl-amino]-3,4-dioxo-cyclobut-1-
enylamino}-3-phenyl-propionic acid;
N-[3,4-Dioxo-2-(4-phenyl-piperazin-1-yl)-cyclobut-1-en-1-yl]-L-phenyl-
alanine;

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(S)-2-[2-(4-Acetyl-piperazin-1-yl)-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid;

(S)-3-(4-Benzoylamino-phenyl)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-propionic acid;

5 (S)-3-(1-Benzyl-1H-imidazol-4-yl)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-propionic acid;

N-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-O-(3-dimethylamino-propyl)-L-tyrosine;

N-[2-[Methyl[2-(4-pyridinyl)ethyl]amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine;

10 N-[2-[Methyl(2-phenylethyl)amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine;

N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine;

15 N-[2-(Methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-4-carbonyl)-amino]-L-phenylalanine;

N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine;

N-[2-[Methyl[2-(4-pyridinyl)ethyl] amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine;

20 N-[2-(Methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-3-carbonyl)-amino]-L-phenylalanine;

N-{2-[Methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enyl}-L-phenylalanine;

25 N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-{4- [4-(N- carboxy-benzoyl)piperidinylcarbonyl]amino}-L-phenylalanine methyl ester;

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid;

(2S)-2-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-(4-dimethyl-30 carbamoyloxy-phenyl)-propionic acid;

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid;

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(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-{2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino}-propionic acid;

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid;

5 (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-{2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino}-propionic acid; and

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-{2-dihexylamino}-3,4-dioxo-cyclobut-1-enylamino}-propionic acid; or a pharmaceutical salt thereof.

10 "Alkyl" as used herein means a branched or straight chain having from 1 to 10 carbon atoms and more preferably from 1 to 8 carbon atoms. Exemplary alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl and hexyl. Alkyl may be substituted and unsubstituted.

15 "Aryl" as used herein means mono or bicyclic aromatic ring having from 5 to 12 carbon atoms. Monocyclic rings preferably have 5 or 6 members and bicyclic rings preferably have 8, 9 or 10 membered ring structures. Exemplary aryl groups include phenyl and naphthyl. Aryl may be substituted or unsubstituted.

20 "Aralkyl" as used herein means an aryl-alkyl group in which the aryl and alkyl group are previously defined. Exemplary aralkyl groups include benzyl and phenethyl. The aralkyl may be substituted or unsubstituted.

"Halogen" is chlorine, fluorine, iodine or bromine.

25 "Heteroaryl" whether used alone or as part of a group such as "heteroaralkyl" means 5 to 10 membered mono or bicyclic aromatic ring having from 1 to 3 heteroatoms selected from N, O and S. Exemplary heteroaryls include pyridyl, pyrazinyl, pyridazinyl, pyrimidinyl, indolyl, imidazolyl, pyrazolyl and pyrrolyl.

30 Preferred heteroaryl groups include 1H-indoly-3-yl, pyridin-3-yl, pyridin-4-yl, and 1H-imidazol-4-yl. The heteroaryl may be substituted or unsubstituted.

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“Heteraralkyl” means an heteroaryl-alkyl group in which the heteroaryl and alkyl are as previously described. Exemplary heteroaralkyls include pyridylmethyl, pyridylethyl, thienylethyl, thienylmethyl, indolylmethyl, and furylmethyl. The heteraralkyl may be substituted or unsubstituted.

5

Heterocycloalkyl refers to a monocycloalkyl having from 5 to 10 members including one or more heteroatoms selected from N, O or S. The heterocycloalkyl may be saturated or unsaturated and may be substituted or unsubstituted.

10

Suitable substituents, unless otherwise noted are unsubstituted and include, but are not limited to, alkyl of 1 to 3 carbon atoms, halogen, -CN, -NO₂, perhaloalkyl of 1 to 3 carbon atoms, aryl, aralkyl, -NR⁴COR⁵, -CO₂R⁴, -OR⁴, -OCONR⁶R⁷ or -O(CH₂)_mNR⁶R⁷ wherein R⁴ is hydrogen, alkyl of 1 to 3 carbon atoms, or aralkyl of 7-10 carbon atoms, R⁵ is aryl, heteroaryl or heterocycloalkyl, R⁶ and R⁷ are independently, hydrogen or alkyl of 1 to 3 carbon atoms, or R⁶ and R⁷, taken together may form a heterocycloalkyl, and m is an integer from 1 to 6.

Carbon number refers to the number of carbons in the carbon backbone and does not include carbon atoms occurring in substituents thereof.

20

Where terms are used in combination, the definition for each individual part of the combination applies unless defined otherwise.

25

Pharmaceutically acceptable salts are the acid addition salts which can be formed from a compound of the above general formula and a pharmaceutically acceptable acid such as phosphoric, sulfuric, hydrochloric, hydrobromic, citric, maleic, succinic, fumaric, acetic, lactic, nitric, sulfonic, p-toluene sulfonic, methane sulfonic acid, and the like.

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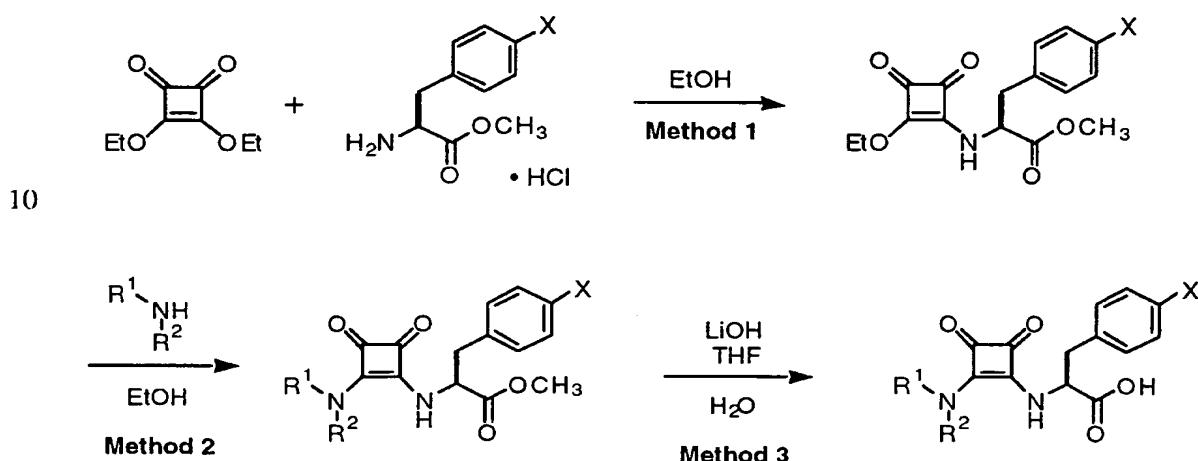
The compounds of this invention contain a chiral center, providing for various seteroisomeric forms of the compounds such as racemic mixtures as well as the individual optical isomers. The individual isomers can be prepared directly or by

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asymmetric or stereospecific synthesis or by conventional separation of optical isomers from the racemic mixture.

Novel compounds of Formula I are prepared by the sequential addition of appropriate amine nucleophiles to 3,4-diethoxy-3-cyclobutene-1,2-dione in alcoholic solvent, followed by hydrolysis of the precursor carboxylic acid ester to the parent acid by treatment with aqueous base as shown in the following reaction schemes.

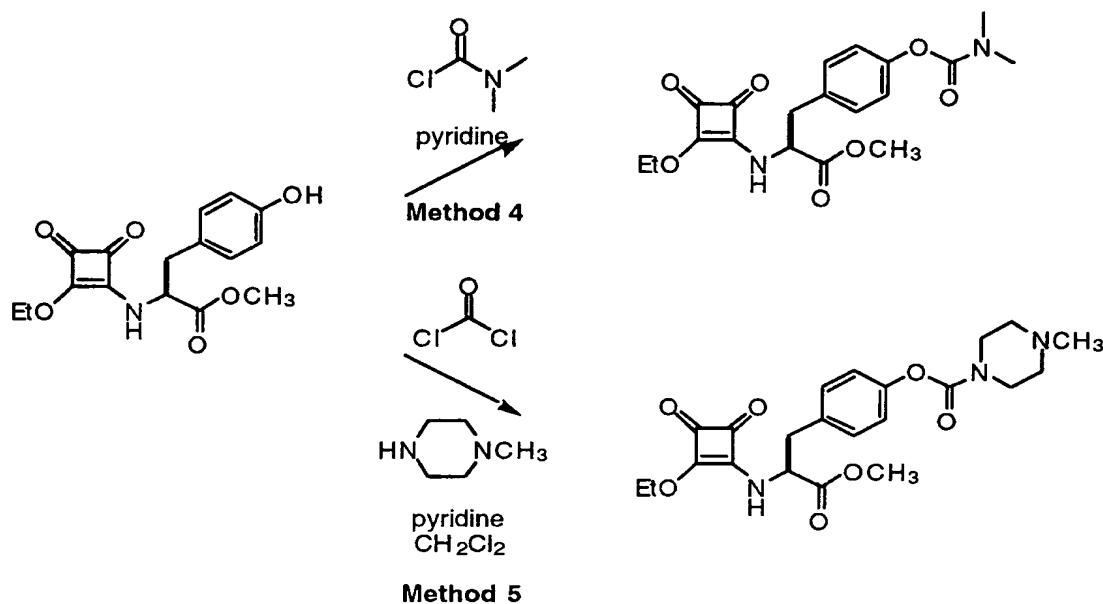
Scheme 1



15 In the case where the product of Method 1 contains $X = OH$, the corresponding carbamates are prepared as in Scheme 2.

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Scheme 2



5 The carbamate products are then further elaborated via Method 2 above.

The method of preparing compounds of Formula I as described above are exemplified in the following specific examples. These examples are illustrative and are not meant to be limiting to this disclosure in any way. Other methods of preparing compounds of the present invention may be apparent to those skilled in the art. Reactants and reagents used are either commercially available or can be prepared according to standard literature procedures.

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Example 1 (Method 1)

[2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester

To a stirred solution of L-phenylalanine methyl ester HCl (2.0 mmol, 431 mg) in
5 absolute ethanol (20 mL) was added triethylamine (2.2 mmol, 202 mg; 278 μ L) and
the resulting solution was stirred at room temperature for 15 minutes. Subsequently,
neat 3,4-diethoxy-3-cyclobutene-1,2-dione (2 mmol, 340 mg; 296 μ L) was added
dropwise and the resulting solution was stirred at room temperature overnight, during
which a white solid precipitated out of solution. The volatiles were removed *in vacuo*
10 and the residue was taken up in EtOAc and partitioned between EtOAc and water.
The organics were dried (Na_2SO_4) and purified by flash chromatography (SiO_2 : 1)
20% EtOAc/hexane; 2) 30% EtOAc/hexane; 3) 40% EtOAc/hexane) to afford the
title compound as a colorless oil (551 mg; 85%).

15 ^1H NMR (DMSO-d₆, 300 MHz) δ 9.1 (br d, 1H), 7.24 (m, 6H), 4.88 (m, 1H), 4.55
(m, 3H), 4.16 (m, 2H), 3.23 (dd, 1H), 2.96 (m, 1H), 1.30 (m, 2H).

Example 2 (Method 2)

[2-(Benzylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester

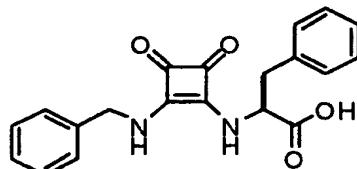
20 To a stirred solution of [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine
methyl ester (0.33 mmol, 100 mg) in absolute ethanol (3 mL) was added neat
benzylamine (0.36 mmol, 39 mg; 40 μ L) dropwise at room temperature. The
resulting solution was stirred at room temperature overnight, during which a white
25 solid precipitated out of solution. The volatiles were removed *in vacuo* and the
residue was taken up in EtOAc and partitioned between EtOAc and water. The
organics were dried (Na_2SO_4), concentrated *in vacuo* and purified by flash
chromatography (SiO_2 : EtOAc/hexane) to afford the title compound as a white solid
(113 mg; 94%).

30 ^1H NMR (DMSO-d₆, 400 MHz) δ 7.85 (br s, 1H), 7.66 (br s, 1H), 7.37 (m, 2H), 7.26
(m, 6H), 7.13 (m, 2H), 5.1 (m, 1H), 4.68 (m, 2H), 3.68 (s, 3H), 3.16 (dd, 1H, J =
13.9, 5.4 Hz), 3.03 (m, 1H).

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Example 3 (Method 3)

[2-(Benzylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine



5

To a stirred solution of [2-(benzylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester (0.16 mmol, 60 mg) in THF (5 mL) was added aqueous LiOH (1.0 M; 0.16 mmol; 160 μ L) and the resulting solution was stirred at room temperature for 3 hours. The volatiles were removed *in vacuo* and the residue 10 partitioned between 0.1M acetic acid and EtOAc. The organics were dried (Na_2SO_4) and concentrated *in vacuo* to afford the title compound as a white solid, mp = 215-216°C (33 mg; 59%).

¹H NMR (DMSO-d₆, 400 MHz) δ 13.1 (br s, 1H), 7.89 (br s, 1H), 7.59 (br s, 1H), 15 7.37 (m, 2H), 7.26 (m, 6H), 7.14 (m, 2H), 4.91 (m, 1H), 4.68 (m, 2H), 3.17 (m, 1H), 3.01 (m, 1H).

MS (EI, m/e (%)) 350 (17, M⁺), 259 (16), 91 (100).

20 **Example 4 (Method 2)**

[2-(benzhydrylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester

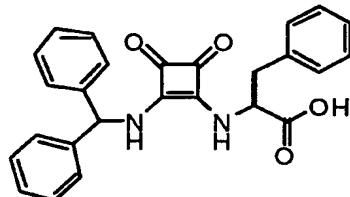
Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and diphenyl-25 methylamine in 88% yield.

¹H NMR (DMSO-d₆, 400 MHz) δ 8.38 (br s, 1H), 7.68 (br s, 1H), 7.39 (m, 4H), 7.26 (m, 9H), 7.12 (m, 2H), 6.33 (m, 1H), 5.01 (m, 1H), 3.69 (s, 3H), 3.16 (dd, 1H, J = 13.4; 5.6 Hz), 3.05 (dd, 1H, J = 13.4; 5.6 Hz).

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Example 5 (Method 3)

[2-(benzhydrylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine



5 Following the procedure of Method 3 above, the title compound was obtained in 76% yield as a white solid, mp = 187-188°C.

¹H NMR (DMSO-d₆, 400 MHz) δ 13.2 (br s, 1H), 8.41 (br s, 1H), 7.62 (br s, 1H), 7.38 (m, 4H), 7.30 (m, 2H), 7.24 (m, 7H), 7.13 (d, 2H, J = 7.02 Hz), 6.34 (m, 1H), 10 4.90 (M, 1H), 3.16 (dd, 1H, J = 13.7; 4.9 Hz), 3.05 (m, 1H).
 MS ((+)FAB, m/e (%)) 449 (14, (M+Na)⁺), 427 (45, (M+H)⁺), 217 (33), 167 (100).
 IR (KBr, cm⁻¹) 3200, 1790, 1730, 1640, 1575, 1530, 1440, 710.

Anal. Calc'd for C₂₆H₂₂N₂O₄ • 0.25 H₂O: C, 72.45; H, 5.26; N, 6.50.

15 Found: C, 72.69; H, 5.22; N, 6.67.

Example 6 (Method 2)

2-[2-(1H-Indol-3-yl)-ethylamino]-3,4-dioxo-cyclobut-1-enylamino-L-phenylalanine methyl ester

20 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and tryptamine in 86% yield.

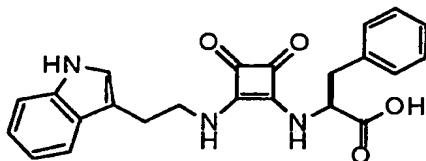
25 ¹H NMR (DMSO-d₆, 400 MHz) δ 10.88 (s, 1H), 7.68 (br s, 1H), 7.57 (d, 2H, J = 7.8 Hz), 7.33 (d, 1H, J = 7.8 Hz), 7.23 (m, 3H), 7.12 (m, 3H), 7.07, (dt, 1H, J = 7.03; 1.1 Hz), 6.96 (dt, 1H, J = 7.03; 1.1 Hz), 5.0 (m, 1H), 3.79 (m, 2H), 3.67 (s, 3H), 3.13 (M, 1H), 3.02 (m, 1H), 2.91 (m, 2H).

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Example 7 (Method 3)

2-[2-[2-(1H-Indol-3-yl)-ethylamino]-3,4-dioxo-cyclobut-1-enylamino]-L-phenylalanine

5



Following the procedure of Method 3 above, the title compound was obtained in 61% yield as a white solid.

10 ¹H NMR (DMSO-d₆, 400 MHz) δ 13.3 (br s, 1H), 10.8 (s, 1H), 7.58 (d, 2H, J = 7.8 Hz), 7.32 (d, 1H, J = 8.1 Hz), 7.10 - 7.30 (m, 6H), 7.06 (m, 1H), 6.97 (m, 1H), 4.90 (m, 1H), 3.79 (m, 2H), 3.38 (q, 1H, J = 7.0 Hz), 3.14 (dd, 1H, J = 13.9; 4.7 Hz), 3.01 (dd, 1H, J = 13.9; 4.7 Hz), 2.92 (m, 2H).
 MS (EI, m/e (%)) 403 (4, M⁺), 385 (16), 294 (60), 143 (100).

15 Anal. Calc'd for C₂₃H₂₁N₃O₄ • 0.4 H₂O: C, 67.27; H, 5.35; N, 10.23.
 Found: C, 67.58; H, 5.82; N, 9.78.

Example 8 (Method 2)

20 2-[3,4-Dioxo-2-[pyridin-3-ylmethyl]-amino]-cyclobut-1-enylamino]-3-phenyl-propanoic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and 3-pyridylmethylamine in 81% yield, mp=191-192°C.

¹H NMR (DMSO-d₆, 400 MHz) δ 8.51 (d, 2H, J = 5.1 Hz), 7.87 (br s, 1H), 7.68 (br s, 2H), 7.40 (dd, 1H, J = 4.7, 7.6 Hz), 7.24 (m, 3H), 7.13 (d, 2H, J = 7.2 Hz), 5.01 (br s, 1H), 4.72 (d, 2H, 5.7 Hz), 3.68 (s, 3H), 3.17 (dd, 1H, J = 5.2, 13.7 Hz), 3.03 (m, 1H).

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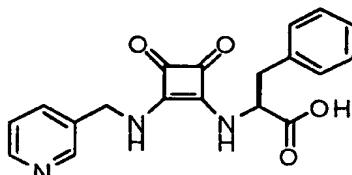
MS (EI, m/e (%)) 365 (6, M⁺), 337 (7), 274 (15), 242 (40), 214 (18), 186 (13), 146 (44), 44 (100).

IR (KBr, cm⁻¹) 3175, 2960, 1800, 1745, 1650, 1570, 1480, 1430, 1310, 1280.

5 Anal. Calc'd for C₂₆H₁₉N₃O₄: C, 65.74; H, 5.24; N, 11.50.
Found: C, 65.22; H, 5.15; N, 11.27.

Example 9 (Method 3)

{3,4-Dioxo-2-[(pyridin-3-ylmethyl)-aminol]-cyclobut-1-enyl}-L-phenylalanine



Following the procedure of Method 3 above, the title compound was obtained in 9% yield as a white solid, mp = 259-261°C.

15 ¹H NMR (DMSO-d₆, 300 MHz) δ 13.25 (br s, 1H), 8.53 (br d, 2H) 7.93 (br s, 1H), 7.69 (br d, 2H), 7.41 (m, 1H), 7.20 (m, 5H), 4.91 (m, 1H), 4.73 (m, 2H), 3.18 (dd, 1H), 3.03 (m, 1H).
MS ((+) FAB, m/e (%)) 352 (10, (M+H)⁺), 232 (17), 179 (23), 157 (100).

20 **Example 10 (Method 2)**

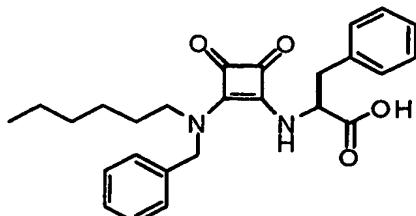
[2-(Benzyl-hexyl-amino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and 25 benzyl(hexyl)-amine in 57% yield as a light yellow oil which was carried on immediately to the subsequent reaction.

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Example 11 (Method 3)

[2-(Benzyl-hexyl-amino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine



5 Following the procedure of Method 3 above, the title compound was obtained in 41% yield as a yellow foam, mp = 61-65°C.

10 ¹H NMR (DMSO-d₆, 400 MHz) δ 13.1 (s, 1H), 7.79 (br s, 1H), 7.35 (dd, 3H, J = 10.6, 6.9 Hz), 7.21 (m, 6H), 5.14 (m, 1H), 4.67 (br s, 2H), 3.38 (br m, 2H), 3.26 (dd, 2H, J = 14, 4.0 Hz), 2.98 (dd, 1H, J = 14.1, 11.2 Hz), 1.38 (br s, 2H), 1.15 (m, 6H), 0.82 (t, 3H, J = 6.8 Hz).

MS ((+)FAB, m/e (%)) 457 (76, (M + Na)⁺), 435 (100, (M + H)⁺), 389 (13), 192 (35).
 IR (KBr, cm⁻¹) 3290, 2940, 1800, 1740, 1675, 1570, 1520, 700.

15 Anal Calc'd for C₂₆H₃₀N₂O₄ • 0.25 H₂O; C, 71.13; H, 7.00; N, 6.38.
 Found: C, 71.31; H, 7.00; N, 6.20.

Example 12 (Method 2)

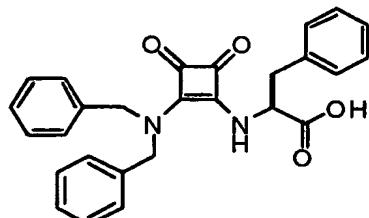
(2-Dibenzylamino-3,4-dioxo-cyclobut-1-enylamino)-L-phenylalanine methyl ester

20 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and dibenzyl-amine in 44% yield as a light yellow solid which was carried on immediately to the subsequent reaction.

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Example 13 (Method 3)

(2-Dibenzylamino-3,4-dioxo-cyclobut-1-enylamino)-L-phenylalanine



Following the procedure of Method 3 above, the title compound was obtained in 79%
5 yield as a yellow solid.

¹H NMR (DMSO-d₆, 400 MHz) δ 13.13 (s, 1H), 8.01 (d, 1H, J = 9.0 Hz), 7.35 (m,
6H), 7.20 (m, 8H), 5.19 (m, 1H), 4.55 (br s, 4H), 3.26 (dd, 2H, J = 3.9, 14.0 Hz),
2.97 (m, 1H).
MS (EI, m/e (%)) 440 (20, M⁺), 349 (16), 91(100).
10 IR (KBr, cm⁻¹) 3450-3250 (br), 2925, 1800, 1740, 1680, 1570, 1520, 1445, 1265,
700.

Anal. Calc'd for C₂₇H₂₄N₂O₄: C, 73.62; H, 5.49; N, 6.36.

Found: C, 72.48; H, 5.41; N, 6.01.

15

Example 14 (Method 2)

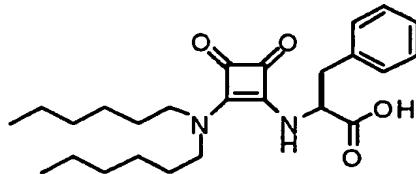
(S)-2-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-phenyl-propionic acid
methyl ester

20 Following the procedure of Method 2 above, the title compound was obtained from
[2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and
dihexylamine in 62% yield as a light yellow solid which was carried on immediately
to the subsequent reaction.

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Example 15 (Method 3)

(S)-2-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-phenyl-propionic acid



5 Following the procedure of Method 3 above, the title compound was obtained in 86% yield as a colorless oil.

¹H NMR (DMSO-d₆, 400 MHz) δ 13.1 (br s, 1H), 7.58 (d, 1H, J = 9.2 Hz), 7.2 (m, 5H), 5.09 (m, 1H), 3.44 (br s, 4H), 3.24 (dd, 1H, J = 4.0, 13.8 Hz), 3.0 (dd, 1H, J = 11.3, 14.1 Hz), 1.43 (br s, 4H), 1.20 (m, 12H), 0.84 (t, 6H, J = 7.0 Hz).
 10 MS (EI, m/e (%)) 428 (100, M⁺), 372 (36), 337 (55), 224 (30).

Example 16 (Method 2)

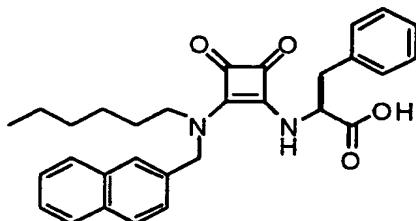
(S)-2-[2-(Hexyl-naphthalen-2-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and (2-naphthalenyl-methyl)hexylamine in 63% yield as a colorless oil which was carried on 20 immediately to the subsequent reaction.

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Example 17 (Method 3)

(S)-2-[2-(Hexyl-naphthalen-2-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid



5

Following the procedure of Method 3 above, the title compound was obtained in 80% yield as a light yellow solid, mp = 62-70°C.

¹H NMR (DMSO-d₆, 400 MHz) δ 13.15 (br s, 1H), 7.91 (m, 2H), 7.80 (s, 1H), 7.52 (m, 2H), 7.29 (m, 2H) 7.21 (m, 6H), 5.17 (m, 1H), 4.84 (br s, 2H), 3.24 (dd, 2H, J = 3.7, 14.3 Hz), 2.99 (m, 1H), 1.48-1.2 (m, 3H), 1.13 (s, 6H), 0.78 (t, 3H, J = 6.7 Hz).
MS (EI, m/e (%)) 484 (5, M⁺), 439 (4), 219 (28), 44 (100).

Example 18 (Method 2)

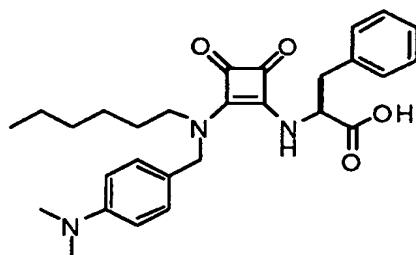
¹⁵ (S)-2-[2-[(4-Dimethylamino-benzyl)-hexyl-amino]-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and (4-dimethyl-
20 aminobenzyl)hexylamine in 59% yield as a colorless oil which was carried on immediately to the subsequent reaction.

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Example 19 (Method 3)

(S)-2-{2-[(4-Dimethylamino-benzyl)-hexyl-aminol]-3,4-dioxo-cyclobut-1-enylamino}-3-phenyl-propionic acid



5

Following the procedure of Method 3 above, the title compound was obtained in 97% yield as a white solid, mp = 77-80°C.

10 ¹H NMR (DMSO-d₆, 400 MHz) δ 13.07 (s, 1H), 7.78 (br s, 1H), 7.23 (m, 5H), 7.02 (br s, 2H), 6.65 (d, 2H, J = 7.9 Hz), 5.16 (br s, 1H), 4.49 (br s, 2H), 3.26 (dd, 1H, J = 3.7, 13.8 Hz), 3.0 (m, 1H), 2.87 (s, 6H), 1.48 (br s, 3H), 1.15 (m, 7H), 0.82 (t, 3H, J = 6.8 Hz).
 MS ((+)FAB, m/e (%)) 500 (100, [M+Na]⁺), 478 (34, [M+H]⁺), 455 (17), 357 (32).

15 **Example 20 (Method 2)**

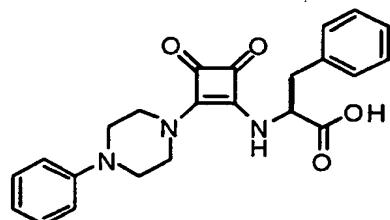
N-[3,4-Dioxo-2-(4-phenyl-piperazin-1-yl)-cyclobut-1-en-1-yl]-L-phenylalanine methyl ester

20 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and 1-phenyl-piperazine in 63% yield as a white solid which was carried on immediately to the subsequent reaction.

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Example 21 (Method 3)

N-[3,4-Dioxo-2-(4-phenyl-piperazin-1-yl)-cyclobut-1-en-1-yl]-L-phenylalanine



5 Following the procedure of Method 3 above, the title compound was obtained in 65% yield as a white solid, mp = 165-167°C.

10 ¹H NMR (DMSO-d₆, 400 MHz) δ 13.1 (br s, 1H), 8.01 (d, 1H, J = 9.0 Hz) 7.29-7.16 (m, 7H), 6.98 (d, 2H, J = 8.1 Hz), 6.82 (t, 1H, J = 7.2 Hz), 5.08 (m, 1H), 3.77 (br s, 4H), 3.24 (dd, 2H, J = 4.0, 14.0 Hz), 3.19 (t, 3H, J = 5.0 Hz), 2.98 (dd, 1H, J = 11.0, 13.8 Hz).
MS (EI, m/e (%)) 405 (48, M⁺), 361 (6), 304 (5), 44 (100).

Example 22 (Method 2)

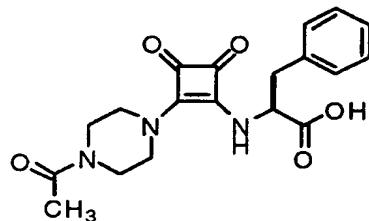
15 (S)-2-[2-(4-Acetyl-piperazin-1-yl)-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and 1-
20 acetylpirperazine in 71% yield as a white solid which was carried on immediately to the subsequent reaction.

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Example 23 (Method 3)

(S)-2-[2-(4-Acetyl-piperazin-1-yl)-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid



5

Following the procedure of Method 3 above, the title compound was obtained in 39% yield as a white solid, mp = 155-158°C.

10 ^1H NMR (DMSO- d_6 , 400 MHz) δ 13.1 (br s, 1H), 7.96 (d, 1H, J = 9.2 Hz) 7.24 (m, 5H), 5.07 (m, 1H), 3.66 (br s, 2H), 3.57 (br s, 3H), 3.50 (d, 3H, J = 4.2 Hz), 3.23 (dd, 1H, J = 4.2, 13.8 Hz), 2.97 (dd, 1H, J = 11.0, 13.8 Hz), 2.03 (s, 3H).
 MS (EI, m/e (%)) 371 (21, M^+), 270 (10).

Example 24 (Method 1)

15 [2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-(4-benzoylamino)phenylalanine methyl ester

20 Following the procedure of Method 1 above, the title compound was obtained from L-(4-benzoylamino)phenylalanine methyl ester hydrochloride and 3,4-diethoxy-3-cyclobutene-1,2-dione in 64% yield.

25 ^1H NMR (DMSO- d_6 , 300 MHz) δ 10.2 (s, 1H), 9.1 (br dd, 1H), 7.93 (dd, 2H), 7.69 (d, 2H), 7.53 (m, 3H), 7.2 (d, 2H), 4.59 (m, 3H), 3.7 (s, 3H), 3.21 (dd, 1H), 2.93 (br m, 1H), 1.31 (m, 3H).

25

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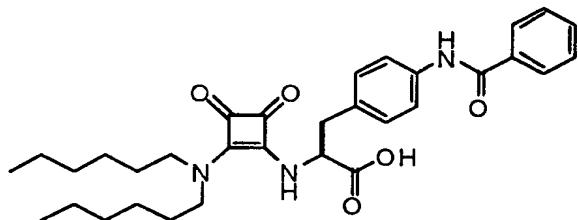
Example 25 (Method 2)

(S)-3-(4-Benzoylamino-phenyl)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-propionic acid methyl ester

5 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-(4-benzoylamino)phenylalanine methyl ester and dihexylamine in 70% yield as a white solid which was carried on immediately to the subsequent reaction.

10 **Example 26 (Method 3)**

(S)-3-(4-Benzoylamino-phenyl)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-propionic acid



15 Following the procedure of Method 3 above, the title compound was obtained in 61% yield as a white solid, mp = 95-100°C.

19 ¹H NMR (DMSO-d₆, 400 MHz) δ 13.1 (br s, 1H), 10.17 (s, 1H), 7.91 (m, 2H), 7.68 (d, 2H, J = 8.6 Hz), 7.6 - 7.48 (m, 4H), 7.19 (d, 2H, J = 8.6 Hz), 5.09 (m, 1H), 3.45 (br m, 4H), 3.22 (dd, 1H, J = 3.8, 13.9 Hz), 2.98 (dd, 1H, J = 11.2, 13.8 Hz), 1.43 (br s, 4H), 1.20 (s, 12H), 0.80 (t, 6H, J = 6.7 Hz).

20 MS ((+)FAB, m/e (%)) 570 (51, [M+Na]⁺), 548 (25, [M+H]⁺), 210 (10), 105 (100).

Anal. Calc'd for C₃₂H₄₁N₃O₅ • 0.4 H₂O: C, 69.26; H, 7.59; N, 7.57.

25 Found: C, 69.14; H, 7.55; N, 7.52.

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Example 27 (Method 1)

[2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-(*im*-benzyl)histidine methyl ester

Following the procedure of Method 1 above, the title compound was obtained from
 5 L-(*im*-benzyl)histidine methyl ester hydrochloride and 3,4-diethoxy-3-cyclobutene-
 1,2-dione in 58% yield.

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¹H NMR (DMSO-d₆, 400 MHz) δ 7.87 (d, J = 8.8 Hz, 1H); 7.66 (s, 1H), 7.29 (m, 3H), 7.16 (dd, J = 6.4, 1.8 Hz, 2H), 6.92 (s, 1H), 5.13 (s, 2H), 5.04 (q, J = 8.3, 5.8 Hz, 1H), 3.55 (br, 4H), 3.01 (m, 2H), 1.49 (br s, 4H), 1.21 (s, 13H), 0.82 (s, 6H).
MS ((+)FAB, m/e (%)) 509 (100, [M + H]⁺), 185 (30), 172 (40).

5

Anal. Calc'd for C₂₉H₄₀N₄O₄ • 0.5 H₂O: C, 67.28; H, 7.98; N, 10.82.
Found: C, 67.53; H, 8.10; N, 10.47.

Example 30 (Method 1)

10 [2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-O-(3-dimethylaminopropyl)-L-tyrosine methyl ester

Following the procedure of Method 1 above, the title compound was obtained from
O-(3-dimethylaminopropyl)-L-tyrosine methyl ester hydrochloride and 3,4-diethoxy-
15 3-cyclobutene-1,2-dione in 71% yield.

¹H NMR (DMSO-d₆, 300 MHz) δ 9.1 (br d, 1H), 7.12 (d, 2H), 6.82 (d, 2H), 4.89 (m, 1H), 4.6 (m, 2H), 3.93 (t, 2H), 3.69 (s, 3H), 3.1 (m, 1H), 2.88 (m, 1H), 2.31 (t, 2H), 2.12 (s, 6H), 1.8 (m, 2H), 1.3 (m, 3H).

20

Example 31 (Method 2)

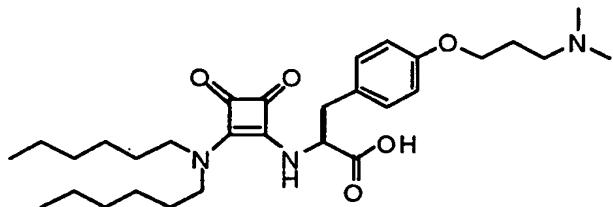
N-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-O-(3-dimethylamino-propyl)-L-tyrosine methyl ester

25 Following the procedure of Method 2 above, the title compound was obtained from
[2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-O-(3-dimethylaminopropyl)-L-tyrosine methyl ester and dihexylamine in 23% yield as a white solid which was carried on
immediately to the subsequent reaction.

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Example 32 (Method 3 (modified))

N-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-O-(3-dimethylamino-propyl)-L-tyrosine



5

Following a modification procedure of Method 3 above, the lithium salt of the title compound was obtained in 66% yield as a light yellow solid. The modified procedure requires removing the volatiles *in vacuo* from the reaction mixture following completion of ester hydrolysis (usually 3 hours at room temperature), followed by 10 partitioning the reaction mixture between EtOAc and water. The aqueous phase is then lyophilized to afford the lithium salt as an amorphous powder.

¹H NMR (DMSO-d₆, 400 MHz) δ 7.08 (d, 1H, J = 6.6 Hz), 6.95 (d, 2H, J = 8.6 Hz), 6.68 (d, 2H, J = 8.6 Hz), 4.28 (m, 1H), 3.87 (t, 2H, J = 6.4 Hz), 3.3 (br s, 4H), 3.05 (d, 2H, J = 5.1 Hz) 2.31 (t, 2H, J = 7.1 Hz), 2.11 (s, 6H), 1.78 (t, 2H, J = 6.9 Hz), 1.43 (br s, 4H), 1.19 (br m, 12H), 0.82 (t, 6H, J = 7.0 Hz).
 MS ((+)FAB, m/e (%)) 536 (100, [M + Li]⁺), 530 (50, [M+H]⁺).
 IR (KBr, cm⁻¹) 3400, 2960, 2930, 2880, 1800, 1575, 1520, 1240.

20 **Example 33 (Method 1)**

[2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester

Following the procedure of Method 1 above, the title compound was obtained from 25 4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester hydrochloride and 3,4-diethoxy-3-cyclobutene-1,2-dione in 71% yield.

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¹H NMR (DMSO-d₆, 300 MHz) δ 10.47 (s, 1H), 9.12 (br dd, 1H), 8.78 (dd, 2H), 7.83 (d, 2H), 7.69 (d, 2H), 7.23 (d, 2H), 4.9 (br m, 1H), 4.59 (m, 2H), 3.7 (s, 3H), 3.22 (dd, 1H), 2.94 (m, 1H), 1.31 (m, 3H).

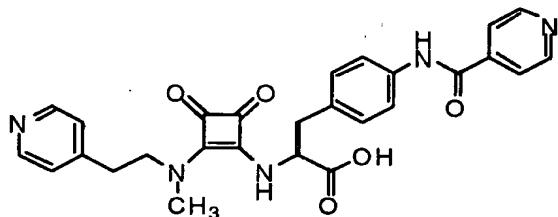
5 **Example 34 (Method 2)**

N-[2-[Methyl[2-(4-pyridinyl)ethyl]amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from
 10 [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and methyl-[2-(4-pyridinyl)ethyl]amine in 41% yield as a yellow foam which was carried on immediately to the subsequent reaction.

Example 35 (Method 3 (modified))

15 N-[2-[Methyl[2-(4-pyridinyl)ethyl]amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine



Following the procedure of Method 3 (modified) above, the title compound was
 20 obtained as its corresponding lithium salt in 73% yield as a yellow solid.

¹H NMR (DMSO-d₆, 400 MHz) δ 10.45 (s, 1H), 8.74 (dd, J = 1.5, 4.6 Hz, 2H), 8.40 (d, J = 4.8 Hz, 2H), 7.81 (d, J = 6.1 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.40 (br m, 1H), 7.20 (d, J = 5.3 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 4.40 (m, 1H), 3.70 (br s, 2H),
 25 3.10 (m, 6H), 2.81 (m, 2H).

MS ((+)FAB, m/e (%)) 506 (100, [M + Li]⁺), 500 (50, [M+H]⁺).

IR (KBr, cm⁻¹) 3400, 1575, 1530, 1410, 1320.

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Anal. Calc'd for $C_{27}H_{24}N_5O_5Li \cdot 3.5 H_2O$: C, 56.98; H, 5.49; N, 12.31.

Found: C, 56.96; H, 5.34; N, 11.82.

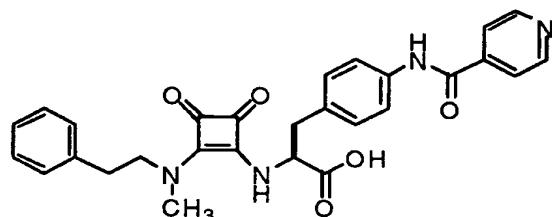
Example 36 (Method 2)

5 N-[2-[Methyl(2-phenylethyl)aminol-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and methyl-(2-phenethyl)amine in 93% yield as a colorless foam which was carried on immediately to the subsequent reaction.

Example 37 (Method 3 (modified))

15 N-[2-[Methyl(2-phenylethyl)aminol-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine



Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 90% yield as a white solid.

20

1H NMR (DMSO-d₆, 400 MHz) δ 10.45 (s, 1H), 8.73 (m, 2H), 7.80 (d, J = 5.7 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.32-7.08 (m, 8H), 4.38 (d, J = 4.6 Hz, 1H), 3.67 (br s, 2H), 3.08 (m, 5H), 2.79 (m, 2H).

MS ((+)FAB, m/e (%)) 521 (100, [M + Na]⁺), 505 (85, [M+Li]⁺), 499 (60, [M+H]⁺).

25 IR (KBr, cm⁻¹) 3400, 1810, 1660, 1580, 1530, 1410, 1330.

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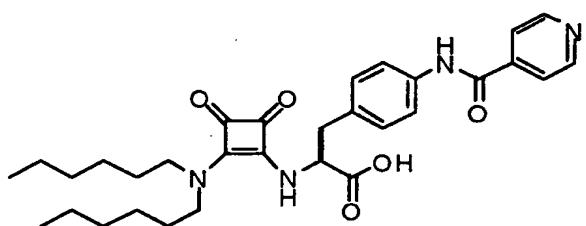
Example 38 (Method 2)

N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from
 5 [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and dihexylamine in 87% yield as a light yellow foam which was carried on immediately to the subsequent reaction.

Example 39 (Method 3 (modified))

10 N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine



Following the procedure of Method 3 (modified) above, the title compound was
 15 obtained as its corresponding lithium salt in 92% yield as a white solid.

¹H NMR (DMSO-d₆, 400 MHz) δ 10.46 (s, 1H), 8.75 (dd, J = 4.4, 1.8 Hz, 2H), 7.83 (dd, J = 4.4, 1.8 Hz, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 6.2 Hz, 1H), 7.05 (d, J = 8.6 Hz, 2H), 4.33 (q, J = 5.5 Hz, 1H), 3.65 (br s, 2H), 3.25 (br s, 2H), 3.11 (d, J = 5.7 Hz, 3H), 1.43 (br s, 4H), 1.15 (br s, 11 Hz), 0.78 (s, 6H).
 20 MS ((+)FAB, m/e (%)) 555 (100, [M+Li]⁺), 549 (97, [M+H]⁺).
 IR (KBr, cm⁻¹) 3330, 2910, 1800, 1660, 1580, 1520, 1410, 1300.

Anal. Calc'd for C₃₁H₃₉N₄O₅Li • 2 H₂O: C, 62.99; H, 7.33; N, 9.48.

25 Found: C, 62.65; H, 7.23; N, 9.31.

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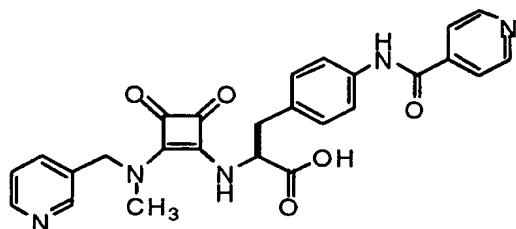
Example 40 (Method 2)

N-[2-(Methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-4-carbonyl)-amino]-L-phenylalanine methyl ester

5 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and methyl-(3-pyridinylmethyl)amine in 88% yield as a colorless foam which was carried on immediately to the subsequent reaction.

10 **Example 41 (Method 3 (modified))**

N-[2-(Methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-4-carbonyl)-amino]-L-phenylalanine



15 Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 85% yield as a yellow solid.

19 ¹H NMR (DMSO-d₆, 400 MHz) δ 10.47 (s, 1H), 8.77 (dd, J = 4.4, 1.8 Hz, 2H), 8.50 (m, 2H), 7.84 (m, 2H), 7.70 (br m, 1H), 7.58 (d, J = 8.6 Hz, 3H), 7.39 (dd, J = 7.6, 4.9 Hz, 1H), 7.11 (d, J = 8.3 Hz, 2H), 4.7 (d, J = 15.2 Hz, 1H), 4.68 (br m, 1H), 4.54 (br s, 1H), 3.18 (dd, J = 13.6, 4.3 Hz, 1H), 3.01 (s, 3H), 2.94 (dd, J = 13.6, 8.3 Hz, 1H).

20 MS ((+), (-) ESI, m/e (%)) 486 (82, [M+H]⁺), 484 (58, [M-H]⁻).

IR (KBr, cm⁻¹) 3400, 1800, 1620, 1580, 1530, 1410, 1325.

Example 42 (Method 1)[2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester

5

Following the procedure of Method 1 above, the title compound was obtained from 4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester hydrochloride and 3,4-diehtoxy-3-cyclobutene-1,2-dione in 74% yield.

10 ^1H NMR (DMSO-d₆, 300 MHz) δ 10.4 (s, 1H), 9.1 (br dd, 1H), 9.08 (d, 1H) 8.75 (dd, 1H), 8.27 (dt, 1H), 7.68 (d, 2H), 7.56 (dd, 1H), 7.23 (d, 2H), 4.9 (m, 1H), 4.58 (m, 2H), 3.68 (s, 3H), 3.23 (dd, 1H), 2.96 (m, 1H), 1.32 (m, 3H).

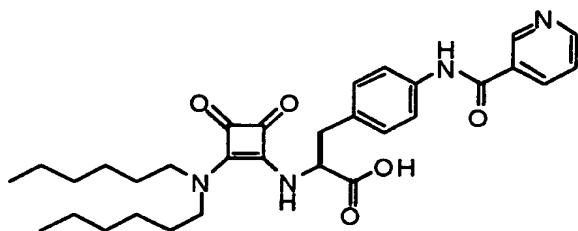
15 **Example 43 (Method 2)**N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from 20 [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and dihexylamine in 95% yield as a yellow foam which was carried on immediately to the subsequent reaction.

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Example 44 (Method 3 (modified))

N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)aminol-L-phenylalanine



5

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 53% yield as a white solid.

¹H NMR (DMSO-d₆, 400 MHz) δ 10.38 (s, 1H), 9.06 (dd, J = 2.4, 1.8 Hz, 1H), 8.72 (dd, J = 4.7, 1.7 Hz, 1H), 8.25 (dt, J = 2.0 Hz, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.54 (m, 1H), 7.09 (d, J = 6.4 Hz, 1H), 7.04 (d, J = 8.6 Hz, 2H), 4.45 (m, 1H), 3.28 (br s, 4H), 3.11 (dd, J = 13.6, 4.8 Hz, 1H), 2.99 (dd, J = 13.8, 7.2 Hz, 1H), 1.40 (br s, 4H), 1.16 (br s, 12H), 0.74 (s, 6H).

15 MS ((+)FAB, m/e (%)) 555 (43, [M+Li]⁺), 549 (100, [M+H]⁺).
 IR (KBr, cm⁻¹) 3375 (br), 2900, 1800, 1660, 1575, 1530, 1410, 1325.

Example 45 (Method 2)

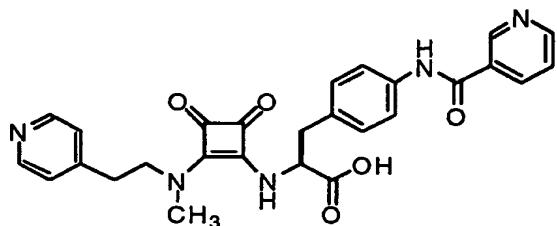
N-[2-[Methyl[2-(4-pyridinyl)ethyl] aminol-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)aminol-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and methyl-[2-(4-pyridinyl)ethyl]amine in 54% yield as a yellow foam which was carried on immediately to the subsequent reaction.

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Example 46 (Method 3 (modified))

N-[2-[Methyl[2-(4-pyridinyl)ethyl] amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine



5

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 81% yield as a pale yellow solid.

¹H NMR (DMSO-d₆, 400 MH) δ 10.39 (s, 1H), 9.04 (d, J = 1.8 Hz, 1H), 8.71 (dd, J = 4.8, 1.8 Hz, 1H), 8.39 (d, J = 4.6 Hz, 2H), 8.24 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 8.3 Hz, 2H), 7.52 (m, 1H), 7.43 (br s, 1H), 7.19 (d, J = 5.3 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 4.42 (d, J = 4.4 Hz, 1H), 3.70 (br s, 2H), 3.10 (m, 5H), 2.81 (m, 2H).
 MS ((+)ESI, m/e (%)) 506 (25, [M+Li]⁺), 500 (100, [M+H]⁺).
 IR (KBr, cm⁻¹) 3400 (br), 1810, 1660, 1580, 1535, 1410, 1320.

15

Anal. Calc'd for C₂₇H₃₄N₅O₅Li • 2.5 H₂O: C, 58.90; H, 5.49; N, 12.72.
 Found: C, 58.68; H, 5.25; N, 12.46.

Example 47 (Method 2)

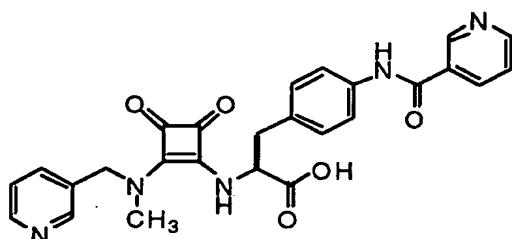
N-[2-(Methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-3-carbonyl)-amino]-L-phenylalanine methyl ester

Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine methyl ester and methyl-(3-pyridinylmethyl)amine in 66% yield as a colorless foam which was carried on immediately to the subsequent reaction.

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Example 48 (Method 3 (modified))

N-[2-(Methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-3-carbonyl)-amino]-L-phenylalanine



5

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 73% yield as a white solid.

10 ^1H NMR (DMSO- d_6 , 400 MHz) δ 10.4 (s, 1H), 9.08 (d, J = 2.2 Hz, 1H), 8.73 (dd, J = 4.8, 1.8 Hz, 1H), 8.5 (m, 2H), 8.28 (dt, J = 2.0 Hz, 1H), 7.72 (br m, 1H), 7.57 (m, 4H), 7.39 (dd, J = 7.7, 4.8 Hz, 1H), 7.12 (d, J = 8.6 Hz, 2H), 4.76 (d, J = 14.9 Hz, 1H), 4.66 (br m, 1H), 4.55 (br s, 1H), 3.18 (dd, J = 13.6, 4.3 Hz, 1H), 3.01 (s, 3H), 2.94 (dd, J = 13.8, 8.2 Hz, 1H).
 15 MS ((-), (+)ESI, m/e (%)) 486 (18, [M+H] $^+$), 484 (100, [M-H] $^-$)
 IR (KBr, cm^{-1}) 3275 (br), 1800, 1660, 1580, 1530, 1410, 1315.

Anal. Calc'd for $\text{C}_{26}\text{H}_{22}\text{N}_5\text{O}_5\text{Li} \cdot 2.8 \text{ H}_2\text{O}$: C, 57.62; H, 5.13; N, 12.92.

Found: C, 57.56; H, 4.74; N, 12.73.

20 **Example 49 (Method 2)**

N-[2-[Methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester

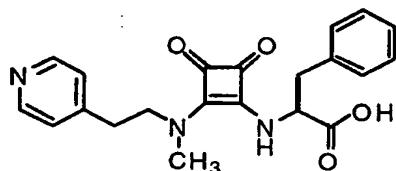
25 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine methyl ester and methyl-[2-(4-pyridinyl)ethyl]amine in 55% yield as a clear oil which was carried on immediately to the subsequent reaction.

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Example 50 (Method 3 (modified))

N-(2-[Methyl-(2-pyridin-4-yl-ethyl)-aminol]-3,4-dioxo-cyclobut-1-enyl)-L-phenylalanine

5



Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 87% yield as a white solid.

10 ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.4 (d, J = 5.7 Hz, 2H), 7.42 (br m, 1H), 7.2 (d, J = 5.7 Hz, 2H), 7.11 (m, 5H), 4.39 (d, J = 4.6 Hz, 1H), 3.68 (br s, 2H), 3.18 (m, 5H), 2.78 (m, 2H).

MS ((+))FAB, m/e (%) 402 (45, $[\text{M}+\text{Na}]^+$), 380 (100, $[\text{M}+\text{H}]^+$).

IR (KBr, cm^{-1}) 3375, 1800, 1580, 1530, 1410.

15

Anal. Calc'd for $\text{C}_{21}\text{H}_{20}\text{N}_3\text{O}_4\text{Li} \cdot 1.5 \text{ H}_2\text{O}$: C, 61.21; H, 5.63; N, 10.20.

Found: C, 61.00; H, 5.44; N, 10.05.

Example 51 (Method 1)

20 [2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-{4-[4-(N-carboxybenzoyl)piperidinylcarbonyl]amino}-L-phenylalanine methyl ester

Following the procedure of Method 1 above, the title compound was obtained from {4-[4-(N-carboxybenzoyl)piperidinylcarbonyl]amino}-L-phenylalanine methyl

25 ester hydrochloride and 3,4-diethoxy-3-cyclobutene-1,2-dione in 37% yield.

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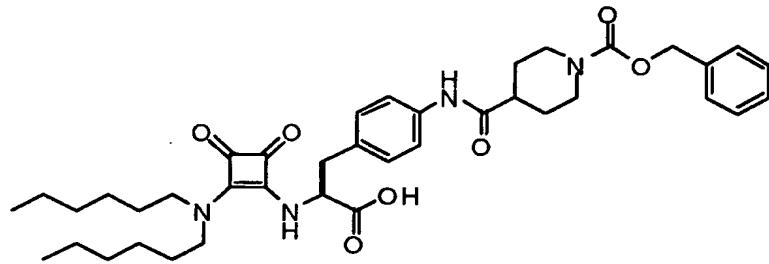
Example 52 (Method 2)

N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]- {4- [4-(N-carboxybenzoyl)piperidinylcarbonyl]amino }-L-phenylalanine methyl ester

5 Following the procedure of Method 2 above, the title compound was obtained from [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]- {4- [4-(N-carboxybenzoyl)piperidinylcarbonyl]-amino }-L-phenylalanine methyl ester and dihexylamine in 50% yield as a clear oil which was carried on immediately to the subsequent reaction.

10 **Example 53 (Method 3)**

N-[2-(Dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]- {4- [4-(N-carboxybenzoyl)-piperidinylcarbonyl]amino }-L-phenylalanine methyl ester



15 Following the procedure of Method 3 above, the title compound was obtained in 75% yield as a light yellow solid, mp = 75-80°C.

¹H NMR (DMSO-d₆, 400 MHz) δ 13.01 (br s, 1H), 9.83 (s, 1H), 7.57 (d, 1H, J = 9.0 Hz), 7.47 (d, 2H, J = 8.3 Hz), 7.33 (m, 4H), 7.12 (d, 2H, J = 8.6 Hz), 5.07 (s, 2H), 5.03 (m, 1H), 4.04 (d, 2H, J = 13.2 Hz), 3.5 (br m, 4H), 3.28 (br s, under H₂O, 1H), 3.16 (dd, 1H, J = 3.8 Hz), 2.93 (dd, 1H, J = 11.0 Hz), 2.85 (br m, 2H), 1.76 (m, 2H), 1.48 (m, 6H), 1.20 (br s, 13H), 0.82 (t, 6H, J = 6.7 Hz).
 MS ([M+H]⁺, m/e (%)) 689 (30), 555 (25), 186 (65), 91 (100).
 IR (KBr, cm⁻¹) 3320, 2930, 1810, 1675, 1580, 1520, 1235.

25

Anal. Calc'd for C₃₉H₅₂N₄O₇. C, 68.00; H, 7.61; N, 8.13.

Found: C, 67.60; H, 7.79; N, 7.95.

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Example 54 (Method 1)

[2-Ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-tyrosine methyl ester

Following the procedure of Method 1 above, the title compound was obtained from
5 L-tyrosine methyl ester hydrochloride and 3,4-diethoxy-3-cyclobutene-1,2-dione in
95% yield.

Example 55 (Method 4)

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-ethoxy-3,4-dioxo-cyclobut-1-

10 enylaminol-propionic acid methyl ester

To a solution of [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-tyrosine methyl ester (1.6 mmol, 500 mg) in pyridine (15 mL) was added neat dimethylcarbamyl chloride (4.7 mmol, 505 mg; 433 μ L) dropwise and the resulting solution was heated at 40°C for
15 18 hours. The volatiles were removed *in vacuo* and the residue was partitioned between EtOAc and 1N HCl. The organics were washed with additional 1N HCl, water, and brine and dried (Na_2SO_4). Purification by flash chromatography (SiO₂: 60% EtOAc/hexane) afforded the title compound as a yellow foam (282 mg; 45% yield).

20

Example 56 (Method 2)

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-
-cyclobut-1-enylaminol-propionic acid methyl ester

25 Following the procedure of Method 2 above, the title compound was obtained from (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-ethoxy-3,4-dioxo-cyclobut-1-enylaminol-propionic acid methyl ester and methyl-(2-phenethyl)amine in 75% yield as a colorless foam which was carried on immediately to the subsequent reaction.

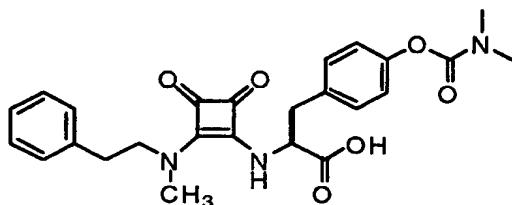
30 ¹H NMR (DMSO-d₆, 400 MHz) δ 7.85 (d, 1H, J = 9.0 Hz), 7.22 (m, 8H), 7.01 (d, 2H, J = 8.6 Hz), 5.12 (m, 1H), 3.72 (br m, 1H), 3.68 (s, 3H), 3.21 (dd, 1H, J = 4.5 Hz), 3.09 (s, 3H), 2.99 (s, 4H), 2.88 (s, 3H), 2.81 (m, 2H).

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Example 57 (Method 3 (modified))

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid

5



Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 82% yield as a white solid.

10 ^1H NMR (DMSO- d_6 , 400 MHz) δ 7.33 (br m, 1H), 7.26 (m, 5H), 7.1 (d, 2H, J = 8.6 Hz), 6.88 (d, 2H, J = 8.6 Hz), 4.36 (d, 1H, J = 4.8 Hz), 3.65 (br m, 2H), 3.12 (dd, 2H, J = 5.1 Hz), 3.07 (s, 3H), 2.98 (s, 3H), 2.86 (s, 3H), 2.79 (m, 2H).
 MS ((+)FAB, m/e (%)) 488 (55, $[\text{M} + \text{Na}]^+$), 472 (60, $[\text{M} + \text{Li}]^+$), 466 (100, $[\text{M} + \text{H}]^+$).

15 IR (KBr, cm^{-1}) 3410, 2910, 1810, 1725, 1580, 1530, 1410, 1210.

Anal. Calc'd for $\text{C}_{25}\text{H}_{26}\text{N}_3\text{O}_6\text{Li} \cdot 1.5 \text{ H}_2\text{O}$. C, 60.24; H, 5.86; N, 8.43.
 Found: C, 60.40; H, 5.65; N, 8.27.

20 **Example 58 (Method 2)**

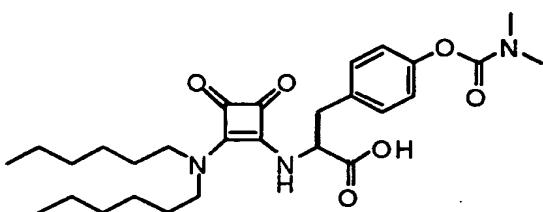
(2S)-2-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-(4-dimethylcarbamoyloxy-phenyl)-propionic acid methyl ester

25 Following the procedure of Method 2 above, the title compound was obtained from (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-ethoxy-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester and dihexylamine in 40% yield as a yellow oil which was carried on immediately to the subsequent reaction.

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Example 59 (Method 3)

(2S)-2-(2-Dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-(4-dimethylcarbamoyloxy-phenyl)-propionic acid



5

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 70% yield as a light yellow solid.

10 ¹H NMR (DMSO-d6, 400 MHz) δ 7.11 (d, 1H, J = 6.4 Hz), 7.045 (m, 2H), 6.87 (m, 2H), 4.28 (m, 1H), 3.5 (br m, 4H), 3.12 (d, 2H, J = 5.3 Hz), 2.99 (s, 3H), 2.87 (s, 3H), 1.43 (br m, 4H), 1.18 (br m, 12H), 0.82 (t, 6H, J = 6.9 Hz).
 MS ((+)ESI, m/e (%)) 533 (30, (M+NH₄)⁺), 516 (100, (M+H)⁺).
 IR (KBr, cm⁻¹) 3400, 2910, 1800, 1730, 1580, 1520, 1380, 1220.

15 Anal. Calc'd for C₂₈H₄₀N₃O₆Li • 1.25 H₂O: C, 61.77; H, 7.87; N, 7.72.
 Found: C, 61.67; H, 7.42; N, 7.45.

Example 60 (Method 2)

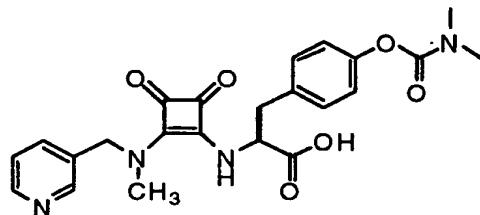
(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enylaminol-propionic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-ethoxy-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester and methyl-(3-pyridinylmethyl)amine in 82% yield as a colorless foam which was carried on immediately to the subsequent reaction.

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Example 61 (Method 3 (modified))

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid



5

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 88% yield as a light yellow solid.

10 ¹H NMR (DMSO-d₆, 400 MHz) 8.50 (m, 2H), 7.67 (br m, 1H), 7.61 (d, 1H, J = 7.7 Hz), 7.38 (dd, 1H, J = 4.7 Hz), 7.12 (d, 2H, J = 8.6 Hz), 6.88 (d, 2H, J = 8.3 Hz), 4.0 (m, 2H), 4.51 (br s, 1H), 3.18 (dd, 1H, J = 4.3 Hz), 3.02 (s, 3H), 2.99 (s, 3H), 2.94 (m, 1H), 2.88 (s, 3H).
 MS ((+)ESI, m/e (%)) 459 (19, [M+Li]⁺), 453 (100, [M+H]⁺).
 IR (KBr, cm⁻¹) 3410, 2920, 1810, 1730, 1580, 1530, 1410, 1220.

15

Anal. Calc'd for C₂₃H₂₃N₄O₆Li • 1.5 H₂O. C, 56.90; H, 5.40; N, 11.54.
 Found: C, 56.63; H, 5.17; N, 11.41.

Example 62 (Method 2)

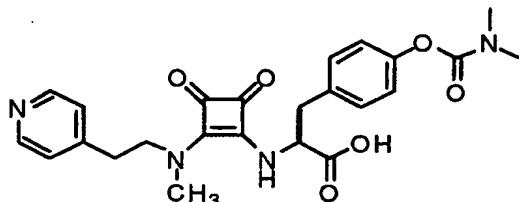
20 (2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-[2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-ethoxy-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester and methyl-[2-(4-pyridinyl)ethyl]amine in 77% yield as a colorless foam which was carried on immediately to the subsequent reaction.

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Example 63 (Method 3 (modified))

(2S)-3-(4-Dimethylcarbamoyloxy-phenyl)-2-{2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino}-propionic acid



5

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 88% yield as a yellow solid.

10 ¹H NMR (DMSO-d₆, 400 MHz) δ 8.41 (d, 2H, J = 5.5 Hz), 7.41 (m, 1H), 7.21 (d, 2H, J = 5.7 Hz), 7.09 (d, 2H, J = 8.6 Hz), 6.88 (d, 2H, J = 8.6 Hz), 4.37 (m, 1H), 3.70 (br m, 2H), 3.09 (m, 5H), 2.98 (s, 3H), 2.86 (s, 3H), 2.80 (br m, 2H).
 MS ((+)ESI, m/e (%)) 473 (20, [M+Li]⁺), 467 (100, [M+H]⁺).
 IR (KBr, cm⁻¹) 3410, 2930, 1800, 1770, 1580, 1530, 1410, 1210, 1160.

15 Anal. Calc'd for C₂₄H₂₅N₄O₆Li • 2.0 H₂O. C, 56.69; H, 5.75; N, 11.02.
 Found: C, 56.82; H, 5.43; N, 10.89.

Example 64 (Method 5)

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-ethoxy-3,4-dioxo-cyclobut-1-enylaminol-propionic acid methyl ester

To a solution of phosgene (1.9M solution in toluene; 7.8 mmol; 4.1 mL) in CH₂Cl₂ (80mL) at 0°C was added a solution of [2-ethoxy-3,4-dioxo-cyclobut-1-enyl]-L-tyrosine methyl ester (7.8 mmol, 2.5 g) and pyridine (8.0 mmol, 633 mg; 647 μL) in 25 CH₂Cl₂ (10 mL) dropwise over 15 minutes. The resulting solution was stirred at 0°C for 30 minutes and a solution was N-methylpiperazine (11.7 mmol, 1.2 g; 1.3 mL) and pyridine (11.7 mmol, 929 mg; 950 μL) in CH₂Cl₂ (10 mL) was then added dropwise over 30 minutes. The resulting solution was warmed to room temperature

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and stirred overnight. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc and water. The organics were dried (Na_2SO_4), concentrated, and purified by flash chromatography (SiO_2 : 5% Et_3N / EtOAc) to afford the title compound as a colorless foam (1.3g; 37%).

5

Example 65 (Method 2)

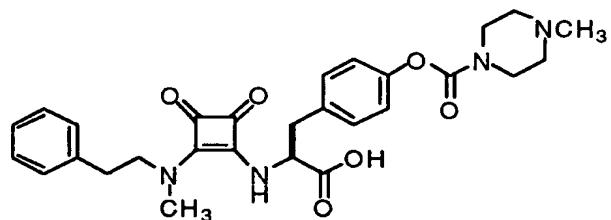
(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylaminol-propionic acid methyl ester

10 Following the procedure of Method 2 above, the title compound was obtained from (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-ethoxy-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester and methyl-(2-phenethyl)amine in 95% yield as a colorless foam which was carried on immediately to the subsequent reaction.

15

Example 66 (Method 3)

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylaminol-propionic acid



20

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 90% yield as a white solid.

25 ^1H NMR (DMSO- d_6 , 400 MHz) δ 7.32-7.16 (m, 6H), 7.09 (d, 2H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.6 Hz), 4.32 (m, 1H), 3.65 (br m, 2H), 3.51 (br s, 2H), 3.38 (br s, 2H), 3.08 (m, 5H), 2.79 (br m, 2H), 2.31 (m, 4H), 2.19 (s, 3H).
 MS (FAB, m/e (%)) 543 (35, ($\text{M}+\text{Na}^+$)), 527 (40, ($\text{M}+\text{Li}^+$)) 521 (100).
 IR (KBr, cm^{-1}) 3410, 2930, 1810, 1725, 1580, 1530, 1410, 1210.

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Anal. Calc'd for $C_{28}H_{31}N_4O_6Li \cdot 2.0 H_2O$. C, 59.78; H, 6.27; N, 9.96.

Found: C, 60.04; H, 6.07; N, 9.77.

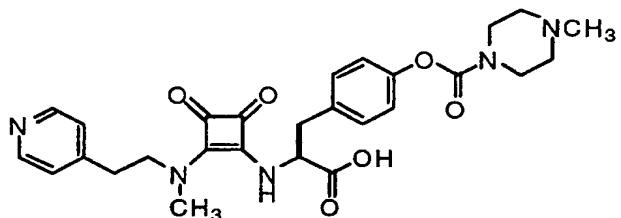
5 **Example 67 (Method 2)**

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-[2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester

Following the procedure of Method 2 above, the title compound was obtained from
 10 (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-ethoxy-3,4-dioxo-cyclobut-1-enylamino]propionic acid methyl ester and methyl-[2-(4-pyridinyl)ethyl]amine in 62% yield as a yellow foam which was carried on immediately to the subsequent reaction.

15 **Example 68 (Method 3 (modified))**

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-[2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino]-propionic acid



20 Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 80% yield as a off-white solid.

¹H NMR (DMSO-d₆, 400 MHz) δ 8.41 (d, 2H, J = 5.9 Hz), 7.39 (m, 1H), 7.21 (d, 2H, J = 5.7 Hz), 7.09 (d, 2H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.6 Hz), 4.38 (m, 1H), 3.69 (br m, 2H), 3.51 (br s, 2H), 3.35 (br s, 4H, under H₂O peak), 3.09 (m, 4H), 2.82 (m, 2H), 2.30 (m, 4H), 2.19 (s, 3H).

25 MS ((+)FAB, m/e (%)) 534 (100, [M+2Li]⁺), 528 (50, [M+Li]⁺).

IR (KBr, cm⁻¹) 3400 (br), 2920, 1810, 1720, 1580, 1530, 1410.

Example 69 (Method 2)

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-{2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino}-propionic acid methyl ester

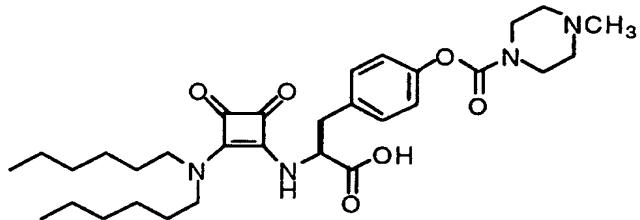
5

Following the procedure of Method 2 above, the title compound was obtained from (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-ethoxy-3,4-dioxo-cyclobut-1-enylamino]-propionic acid methyl ester and dihexylamine in 85% yield as a colorless foam which was carried on immediately to the subsequent reaction.

10

Example 70 (Method 3 (modified))

(2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-{2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino}-propionic acid



15

Following the procedure of Method 3 (modified) above, the title compound was obtained as its corresponding lithium salt in 84% yield as a white solid.

¹H NMR (DMSO-d₆, 400 MHz) δ 7.11 (d, 1H, J = 6.4 Hz), 7.05 (m, 2H), 6.88 (m, 2H), 4.29 (m, 1H), 3.65 (br m, 2H), 3.55 (br s, 3H), 3.34 (br s, 4H, under H₂O peak),

20 3.12 (d, 2H, J = 5.3 Hz), 2.31 (m, 4H), 2.19 (s, 3H), 1.43 (br m, 4H), 1.18 (br m, 11H), 0.82 (t, 6H, J = 6.9 Hz).

MS ((+)FAB, m/e (%)) 577 (100, [M+Li]⁺), 531 (20), 186 (45), 127 (80).

IR (KBr, cm⁻¹) 3410, 2920, 1810, 1580, 1520, 1410.

25 Anal. Calc'd for C₃₁H₄₅N₄O₆Li • 1.5 H₂O. C, 61.68; H, 8.02; N, 9.28.
Found: C, 61.43; H, 7.78; N, 9.04.

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The foregoing compounds were tested for VLA-4 binding activity using the following monovalent FACS assay. The IC_{50} for a compound reflects 50% receptor occupancy. The assay can accurately measure the activity of compounds with IC_{50} ranging from 0.5nM to 1mM.

5

Monovalent FACS Assay for $\alpha_4\beta_1$ Integrin/VCAM-1 Binding

The VLA-4 binding activity of exemplary compounds was measured by measuring the inhibition of the interaction of soluble VCAM-1 with Jurkat cells 10 (ATCC #TIB-153) which express high levels of $\alpha_4\beta_1$ integrin (VLA-4) using a modification of the fluorescence activated cell sorter (FACS) assay described by Yednock, et al., J. Biol. Chem., 1995, 270:28740. VCAM-1 interacts with the cell surface in an $\alpha_4\beta_1$ integrin-dependent fashion.

15 Jurkat cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, streptomycin and glutamine as described by Yednock, *supra*.

Recombinant soluble VCAM-1 (rsVCAM-1) was produced in a baculovirus expression system as a chimeric fusion protein containing the seven immunoglobulin domains of VCAM-1 on the N-terminus and the human IgG₁ heavy chain constant region on the C-terminus as described by Yednock, *supra*. Supernatant containing 20 approximately 10ug/ml rsVCAM-1 was collected after 72 hours and used in the assay without purification.

Jurkat cells (approximately 10^7 cells/ml) were treated with 1.5mM MnCl₂ and 5 μ g/ml 15/7 for 30 minutes on ice to activate β_1 integrin. Mn²⁺ activates the receptor to enhance ligand binding, and 15/7 is a monoclonal antibody that recognizes an 25 activated/ligand occupied conformation of $\alpha_4\beta_1$ integrin and locks the molecule into this conformation thereby stabilizing the VCAM-1/ $\alpha_4\beta_1$ integrin interaction. Yednock, et al., *supra*. Antibodies similar to 15/7 have been prepared and may be used in this assay. For example, *see*, Luque, et al., 1996, J. Bio. Chem., 271: 11067.

Aliquots of 25 μ l cells were incubated for 30 minutes at room temperature 30 with compounds using a standard 5-point serial dilution. 15 μ l of rsVCAM-Fc-

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containing baculovirus supernatant was added to the cells and incubated for 30 minutes on ice as described in Yednock, et al., *supra*.

Cells were washed twice and resuspended in 100 μ l of a 1:100 dilution of FITC-conjugated goat anti-human IgG to detect the human Ig-VCAM-1 construct 5 diluted in assay media containing 2.5% mouse serum to block potential cross-reactivity with cell surface bound 15/7. Cells were incubated on ice for 30 minutes in the dark. Cells were washed twice and analyzed with a standard FACS analysis as described in Yednock, et al., *supra*. on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

10

Data is shown in Table 1.

Table 1

Example	IC50 (FACS) μ M
3	58 μ M
5	101 μ M
7	52 μ M
9	116 μ M
11	4.4 μ M
13	4.4 μ M
15	1.5 μ M
17	36 μ M
19	13 μ M
21	86 μ M
23	40 μ M
26	150 nM
29	631 nM
32	12 nM
35	15 nM
37	2.2 nM
39	1.3 nM
41	26 nM
44	6.5 nM

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Example	IC50 (FACS) μ M
46	40 nM
48	160 nM
50	9 μ M
53	71 nM
57	0.9 nM
59	0.6 nM
61	8.3 nM
63	1.6 nM
66	0.5 nM
68	1.3 nM
70	0.2 nM

Thus, compounds of the present invention exhibit high affinity for VLA-4, and can effectively inhibit the interaction of VLA-4 with VCAM. The compounds are useful

5 for the treatment of inflammatory and autoimmune diseases including, but not limited to multiple sclerosis, meningitis, asthma, Alzheimer's disease, atherosclerosis, AIDS dementia, diabetes, inflammatory bowel syndrome, rheumatoid arthritis, tumor metastasis, tissue transplantation, and myocardial ischemia.

10 The compounds of the present invention can be used in the form of salts derived from pharmaceutically or physiologically acceptable acids or bases. These salts include, but are not limited to, salts with inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid and salts with organic acids such as acetic acid, oxalic acid, succinic acid, and maleic acid. Other salts include salts with

15 alkali metals or alkaline earth metals, such as sodium, potassium, calcium or magnesium. The compounds of the present invention can also be used in the form of esters at the C-terminus; carbamates, amides and the like at the N-terminus or other conventional "pro-drug" forms which, when administered, convert to the active moiety *in vivo*.

Compounds of the present invention may be administered in combination with one or more pharmaceutically acceptable carriers, for example, solvents, diluents and the like. Solid carriers include starch, lactose, dicalcium phosphate, microcrystalline cellulose, sucrose and kaolin, while liquid carriers include sterile water, polyethylene glycols, non-ionic surfactants and edible oils such as corn, peanut and sesame oils. Adjuvants customarily employed in the preparation of pharmaceutical compositions may be advantageously included, such as flavoring agents, coloring agents, preserving agents, and antioxidants, for example, vitamin E, ascorbic acid, BHT and BHA. These compounds may be administered orally as well as by intravenous, intramuscular, or subcutaneous routes. When administered orally in such forms as tablets, capsules, dispersible powders, granules, or suspensions, formulations may contain, for example, from about 0.05 to 5% of suspending agent, syrups containing, for example, from about 10 to 50% of sugar, or elixirs containing, for example, from about 20 to 50% ethanol, and the like. When administration is parenterally, formulation may be, for example, sterile injectable solutions or suspensions containing from about 0.05 to 5% suspending agent in an isotonic medium. Such pharmaceutical preparations may contain, for example, from about 25 to about 90% by weight of active ingredient in combination with a carrier, and more preferably between about 5% and 60% by weight of active ingredient.

20

The preferred pharmaceutical compositions from the standpoint of ease of preparation and administration are solid compositions, particularly tablets and hard-filled or liquid-filled capsules. Oral administration of the compounds is preferred.

25

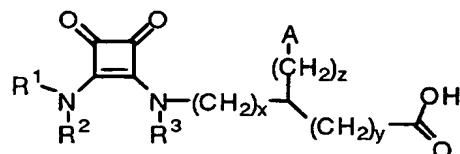
The dosage requirements can be determined by one skilled in the art and will vary with the particular composition employed, the route of administration, the severity of the symptoms presented and the particular subject being treated.

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CLAIMS

What is claimed is:

1. A compound of the formula:



wherein R¹ is alkyl, aryl, heteroaryl, aralkyl or heteroaralkyl;
 R² is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl; or
 R¹ and R² may be taken together to form a saturated or unsaturated
 heterocyclic ring;

10 R³ is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;
 A is aryl or heteroaryl; and
 x, y and z are independently 0, 1, 2, 3,
 or a pharmaceutical salt thereof.

15 2. The compound of Claim 1 wherein A is phenyl, R¹ is alkyl, R² and R³ are H, x
 and y are 0 and z is 1.

3. The compound of Claim 2 wherein A is substituted phenyl.

20 4. The compound of Claim 1 wherein A is phenyl, R¹ is heteroaralkyl, R² and R³
 are H, x and y are 0 and z is 1.

5. The compound of Claim 1 wherein A is substituted aryl and the substituent is
 OCONR⁶R⁷.

25 6. A compound of Claim 1 which is [2-(benzylamino)-3,4-dioxo-cyclobut-1-
 enyl]-L-phenylalanine; or a pharmaceutical salt thereof.

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7. A compound of Claim 1 which is [2-(benzhydrylamino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine; or a pharmaceutical salt thereof.
8. A compound of Claim 1 which is 2-{2-[2-(1H-Indol-3-yl)-ethylamino]-3,4-dioxo-cyclobut-1-enylamino}-L-phenylalanine; or a pharmaceutical salt thereof.
9. A compound of Claim 1 which is {3,4-dioxo-2-[(pyridin-3-ylmethyl)-amino]-cyclobut-1-enyl}-L-phenylalanine; or a pharmaceutical salt thereof.
10. 10. A compound of Claim 1 which is [2-(benzyl-hexyl-amino)-3,4-dioxo-cyclobut-1-enyl]-L-phenylalanine; or a pharmaceutical salt thereof.
11. A compound of Claim 1 which is (2-dibenzylamino-3,4-dioxo-cyclobut-1-enylamino)-L-phenylalanine; or a pharmaceutical salt thereof.
15. 12. A compound of Claim 1 which is (S)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-phenyl-propionic acid; or a pharmaceutical salt thereof.
13. A compound of Claim 1 which is (S)-2-[2-(hexyl-naphthalen-2-ylmethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid; or a pharmaceutical salt thereof.
20. 14. A compound of Claim 1 which is (S)-2-{2-[(4-dimethylamino-benzyl)-hexyl-amino]-3,4-dioxo-cyclobut-1-enylamino}-3-phenyl-propionic acid; or a pharmaceutical salt thereof.
25. 15. A compound of Claim 1 which is N-[3,4-dioxo-2-(4-phenyl-piperazin-1-yl)-cyclobut-1-en-1-yl]-L-phenylalanine; or a pharmaceutical salt thereof.
30. 16. A compound of Claim 1 which is (S)-2-[2-(4-acetyl-piperazin-1-yl)-3,4-dioxo-cyclobut-1-enylamino]-3-phenyl-propionic acid; or a pharmaceutical salt thereof.

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17. A compound of Claim 1 which is (S)-3-(4-benzoylamino-phenyl)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-propionic acid; or a pharmaceutical salt thereof.
- 5 18. A compound of Claim 1 which is (S)-3-(1-benzyl-1H-imidazol-4-yl)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-propionic acid; or a pharmaceutical salt thereof.
- 10 19. A compound of Claim 1 which is N-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-O-(3-dimethylamino-propyl)-L-tyrosine; or a pharmaceutical salt thereof.
20. A compound of Claim 1 which is N-[2-[methyl[2-(4-pyridinyl)ethyl]amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine; or a pharmaceutical salt thereof.
- 15 21. A compound of Claim 1 which is N-[2-[methyl(2-phenylethyl)amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine; or a pharmaceutical salt thereof.
- 20 22. A compound of Claim 1 which is N-[2-(dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(4-pyridinylcarbonyl)amino]-L-phenylalanine; or a pharmaceutical salt thereof.
- 25 23. A compound of Claim 1 which is N-[2-(methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-4-carbonyl)-amino]-L-phenylalanine; or a pharmaceutical salt thereof.
24. A compound of Claim 1 which is N-[2-(dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine; or a pharmaceutical salt thereof.
- 30 25. A compound of Claim 1 which is N-[2-[methyl[2-(4-pyridinyl)ethyl] amino]-3,4-dioxo-1-cyclobuten-1-yl]-4-[(3-pyridinylcarbonyl)amino]-L-phenylalanine; or a pharmaceutical salt thereof.

26. A compound of Claim 1 which is N-[2-(methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enyl]-4-[(pyridine-3-carbonyl)-amino]-L-phenylalanine; or a pharmaceutical salt thereof.

5 27. A compound of Claim 1 which is N-{2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enyl}-L-phenylalanine; or a pharmaceutical salt thereof.

10 28. A compound of Claim 1 which is N-[2-(dihexylamino)-3,4-dioxo-1-cyclobuten-1-yl]- {4- [4-(N- carboxybenzoyl)-piperidinylcarbonyl]amino}-L-phenylalanine methyl ester; or a pharmaceutical salt thereof.

15 29. A compound of Claim 1 which is (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid; or a pharmaceutical salt thereof.

20 30. A compound of Claim 1 which is (2S)-2-(2-dihexylamino-3,4-dioxo-cyclobut-1-enylamino)-3-(4-dimethylcarbamoyloxy-phenyl)-propionic acid; or a pharmaceutical salt thereof.

25 31. A compound of Claim 1 which is (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-[2-(methyl-pyridin-3-ylmethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid; or a pharmaceutical salt thereof.

32. A compound of Claim 1 which is (2S)-3-(4-dimethylcarbamoyloxy-phenyl)-2-{2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino}-propionic acid; or a pharmaceutical salt thereof.

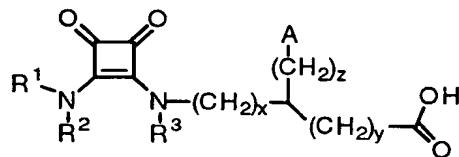
30 33. A compound of Claim 1 which is (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-[2-(methyl-phenethyl-amino)-3,4-dioxo-cyclobut-1-enylamino]-propionic acid; or a pharmaceutical salt thereof.

34. A compound of Claim 1 which is (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-{2-[methyl-(2-pyridin-4-yl-ethyl)-amino]-3,4-dioxo-cyclobut-1-enylamino}-propionic acid; or a pharmaceutical salt thereof.

5

35. A compound of Claim 1 which is (2S)-3-[4-(4-methylpiperazinyl)carbamoyloxy-phenyl]-2-(2-dihexylamino)-3,4-dioxo-cyclobut-1-enylamino}-propionic acid; or a pharmaceutical salt thereof.

10 36. A method for inhibiting leukocyte adhesion in a patient suffering from a condition associated with leukocyte adhesion comprising administering to the patient a therapeutically effective amount of a compound of the formula:



wherein R¹ is alkyl, aryl, heteroaryl, aralkyl or heteroaralkyl;

15 R² is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl; or

R¹ and R² may be taken together to form a saturated or unsaturated heterocyclic ring;

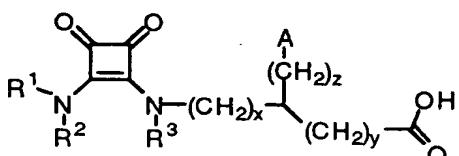
R³ is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

A is aryl or heteroaryl; and

20 x, y and z are independently 0, 1, 2, 3,

or a pharmaceutical salt thereof.

25 37. A method of treating a patient suffering from an inflammatory diseases comprising administering to the patient a therapeutically effective amount of a compound of the formula:



- 54 -

wherein R^1 is alkyl, aryl, heteroaryl, aralkyl or heteroaralkyl;
 R^2 is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl; or
 R^1 and R^2 may be taken together to form a saturated or unsaturated
heterocyclic ring;

5 R^3 is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;
A is aryl or heteroaryl; and
x, y and z are independently 0, 1, 2, 3,
or a pharmaceutical salt thereof.

10 38. The method of Claim 36 wherein the inflammatory disease is selected from the group consisting multiple sclerosis, meningitis, asthma, Alzheimer's disease, atherosclerosis, AIDS dementia, diabetes, inflammatory bowel syndrome, rheumatoid arthritis, tumor metastasis, tissue transplantation, and myocardial ischemia.

15 39. A pharmaceutical composition comprising a compound of the formula:

wherein R^1 is alkyl, aryl, heteroaryl, aralkyl or heteroaralkyl;
 R^2 is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl; or
 R^1 and R^2 may be taken together to form a saturated or unsaturated
20 heterocyclic ring;
 R^3 is H, alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;
A is aryl or heteroaryl; and
x, y and z are independently 0, 1, 2, 3,
and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

Int'l. Application No
PCT/US 99/29369

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C225/20 C07C221/00 C07D209/14 C07D213/38 C07D295/116
C07D295/185 C07D295/205 C07C271/00 C07D213/82 A61K31/13
A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 27947 A (RHONE-POULENC RORER LTD., UK) 8 December 1994 (1994-12-08) claims 1,20	1,36-39
A	WO 98 50347 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, USA) 12 November 1998 (1998-11-12) page 52-55	1,36-39



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of the actual completion of the International search

21 March 2000

Date of mailing of the International search report

31/03/2000

Name and mailing address of the ISA

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Authorized officer

Bader, K

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/29369

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims No.: 36-38
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 36-38
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. Claims No.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. Claims No.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims No.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims No.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members.

International Application No
PCT/US 99/29369

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9427947	A 08-12-1994	AU	6803594 A	20-12-1994
WO 9850347	A 12-11-1998	AU	7273598 A	27-11-1998